

THE BASIS OF SEX INHERITANCE IN SPHÆROCARPOS.

By CHARLES E. ALLEN.

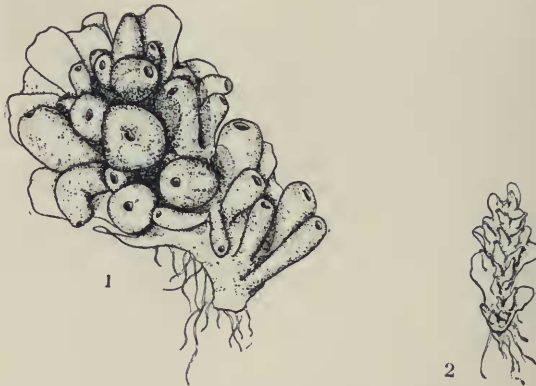
(Read April 25, 1919.)

SPHÆROCARPOS IN CULTIVATION.

The studies here described were made upon cultures of *Sphaerocarpos Donnellii* Aust. growing under greenhouse conditions. Through the kindness of Dr. A. B. Stout, living plants of this species were received from the New York Botanical Garden on January 13, 1916. These plants, as Dr. Stout informed me, had been obtained from Mr. Severin Rapp, Sanford, Florida. To a request for additional material Mr. Rapp very generously responded, as he has to later similar requests, and the greater part of my present cultures of this species began with material which he sent. Plants have been received from Mr. Rapp on February 4 and March 10, 1916, on January 21 and February 23, 1918, and on April 10, 1919. On February 16, 1916, plants of *Sphaerocarpos* were obtained for class use from the Plant Study Company, Cambridge, Massachusetts. A letter from the company informed me that they had been collected at Miami, Florida, by Miss Clara Hart. Some of these plants were used also as starting points for greenhouse cultures. Plants of all the cultures here referred to have been identified as those of *S. Donnellii*, and this identification has been confirmed by Miss Caroline C. Haynes, to whom were sent representatives (including sporophytes) of each set of cultures except those derived from the last lot of plants supplied by Mr. Rapp.

My experience has shown that the thalli of *S. Donnellii*. (and of *S. texanus* Aust. as well) can be kept growing indefinitely, the posterior portions dying as growth continues at the anterior end. This applies to the gametophytes of both sexes, although the male thalli are much more susceptible to unfavorable conditions and it is only with some care and difficulty that cultures of the male plants can be

kept in healthy condition. In mixed cultures the female plants crowd and choke out the males, which latter in such cultures are sure sooner or later to disappear. As already indicated, I have cultures (now purely female) which have been growing continuously since the early months of 1916, the plants having multiplied as a result of their branching and apical growth and of the death of the older portions, as well as by regeneration which occurs freely under a variety of conditions from the lateral lobes, from the body of the thallus, and from the involucre. Sporophytes have been formed and the spores scattered in some of the cultures, in which cultures therefore not all the individuals now living are the result of the vegetative growth of those originally present. It is easy, however, to obviate this possibility, so that a culture (clon or pure line) of any desired extent can be obtained which is known to have been derived by vegetative means from a single gametophyte, or from



Sphærocarpos Donnellii. Living female (FIG. 1) and male (FIG. 2) plants. Received from Sanford, Florida, April 10, 1919; drawn April 15, 1919. A very small portion of the anterior end of each plant (the upper ends in the figures) represents growth since transference to the greenhouse; otherwise the plants are typical of those in nature. Drawings by Miss Martha Engel. $\times 7$.

the germination of a single spore. My oldest male cultures date from spores which were sown June 16, 1916, the plants derived from which were transplanted and have been kept in culture since March 10, 1917.

C. and R. Douin (1917) report having kept female plants of *S. terrestris* (= *S. Michelii* Bell.) and *S. californicus* (= *S. texanus* Aust.) in cultivation for nine or ten months, and conclude that the female thalli "may live indefinitely at a suitable humidity." The male thalli, however, they say, are much less resistant and "disappear little by little in the presence of humidity." Campbell (1896) has noted the appearance of *Sphærocarpos* plants (apparently of both sexes) in a culture of other liverworts grown under glass, and Goebel (1907) describes a culture of female plants which had grown vigorously for two years. So far as I know, these are the only previously published accounts of *Sphærocarpos* in culture.

In my own work, best results have been obtained by growing the plants in pots containing a mixture of about equal parts of clay loam and sand. The pots stand on earthenware plates or enameled metal pans in a Wardian case; a little water is kept in the plates or pans. The soil in the pots is thus constantly moistened from below. Under these conditions contamination of the cultures by other organisms is kept at a minimum, though of course by no means entirely prevented; and, even in case plants of both sexes are growing in the same pot, fertilization does not occur. Fertilization can be brought about when desired by flooding from above with sterilized water a pot containing male and female thalli. The plants must be transplanted occasionally, both to relieve overcrowding and to free the cultures from contamination, especially by blue-green algæ, which are the greatest source of trouble. The only other weeds that cause serious annoyance are mosses, and they are not difficult of exclusion when a culture is once free from them.

Campbell (1896) and C. and R. Douin (1917) have noted that *Sphærocarpos* under greenhouse conditions takes on a form noticeably different from that which it exhibits in nature. Campbell's plants were either (or both) those of *S. californicus* Aust., which Miss Haynes (1910) and the Douins agree is identical with *S. texanus* Aust., or of *S. cristatus*, a California species later separated and described by Howe (1899). The Douins' statements apply to both *S. Michelii* Bell. and *S. texanus*.

The observations of these authors are confirmed by my own on *S. Donnellii*. Figs. 1 and 2 represent female and male plants, re-

spectively, drawn (living) a few days after their receipt from their native habitat (Sanford, Florida), and therefore fairly typical of the wild form. The female and male plants shown in Figs. 3 and 4 were taken from greenhouse cultures nearly three years old. The most conspicuous differences result from the fact that the plants of both sexes, and particularly their vegetative parts, grow more rap-



Sphaerocarpus Donnellii. Living female (FIG. 3) and male (FIG. 4) plants from greenhouse cultures. Rhizoids not shown in Fig. 4. Drawings by Miss Martha Engel. $\times 7$.

idly and more luxuriantly under favorable conditions indoors; the branching is thus more noticeable, and the lateral lobes especially, which in the wild plants are ordinarily insignificant, become, as previous observers have noted, decidedly leaf-like. The involucre surrounding the sex organs become slenderer and often longer, the archegonial involucre being characteristically tubular in the greenhouse form. The orifices of the archegonial involucre are frequently quite wide in plants grown in the greenhouse, though this was not true of most of the involucre of the particular plant shown in Fig. 3. As a result of the greater development of the vegetative parts and the lessened diameter of the involucre, the latter (in both male and female) appear less crowded in the cultivated than in the wild form. Similar modifications appear in cultures of *S. texanus*, as is shown by a comparison of Figs. 5 and 6 (wild form) with Figs. 7 and 8 (greenhouse form). The plants shown in Figs. 5

and 6 were sent from Austin, Texas, by Professor F. McAllister; those shown in Figs. 7 and 8 were from cultures which began with plants received from Professor R. S. Cocks, New Orleans, January 15, 1917, the drawings having been made in February, 1919.

It must be noted, in considering the differences in question, that

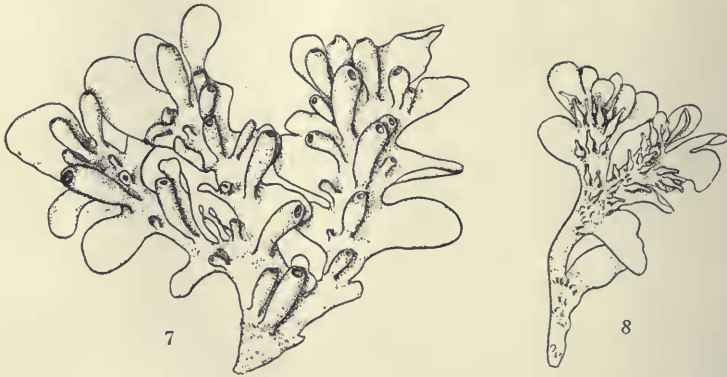


Sphaerocarpos texanus. Living female (FIG. 5) and male (FIG. 6) plants as found in nature. Received from Austin, Texas, February 13, 1919; drawn February 15, 1919. Drawings by Miss Martha Engel. $\times 7$.

in such characters as the size and form of the lateral lobes, size, form, and closeness or distance apart of the involucre, and size of orifice, there is great variation between individual plants in the cultures, as well as evidently also in nature. Compare, for example, Figs. 3 and 9, both representing female plants of *S. Donnellii* from greenhouse cultures, that shown in Fig. 9 bearing sporophytes. Much of this variation is obviously due to environmental conditions, but some of it apparently to the existence of differing strains of sub-specific rank.

The changes that appear under cultivation seem not to be correlated with any loss of function on the part of the sex organs or of the gametes. At any rate, it is easy, as already noted, to secure fertilization, as a result of which sporophytes are formed in abundance, especially in late winter and spring. At other seasons either fertilization or sporophytic development seems to meet with more difficulty, and the proportion of sporophytes obtained is ordinarily smaller.

The sporophytes appearing in the cultures have given rise to spores which were apparently normal in every respect and which germinated, although thus far there has always been a considerable proportion of ungerminated spores. Whether this failure of some of the spores to germinate is due to a lack of viability or to a failure to provide the most favorable conditions for germination is a question yet to be determined. The spores of *S. Donnellii* in my cul-



Sphærocarpos texanus. Living female (FIG. 7) and male (FIG. 8) plants from greenhouse cultures. Rhizoids not shown. Drawings by Miss Martha Engel. $\times 7$.

tures invariably remain united in tetrads (Fig. 11), even to a time when the capsule wall, calyptra, and surrounding involucre have completely broken down and the spores consequently are being scattered.¹ Such a persistent union of the spores is characteristic, as is well known, of other species of *Sphærocarpos*, but not, according to Miss Haynes (1910), of *S. Donnellii* as found in nature.

THE SEX RATIO IN SPHÆROCARPOS.

C. Douin (1909) investigated the sex of the respective members of groups of the thalli of *Sphærocarpos*, the members of each group having resulted presumably from the germination of the spores of a tetrad. His observations showed that, at least as a general rule,

¹ Since this was written, it has been observed in one culture, containing numerous sporophytes, that the tetrad walls had largely broken down, allowing the spores to become separated from one another.

two of the four spores of a tetrad develop into female plants and the other two into male plants. In 9 cases of 81 studied, more than four plants were present in a single group; but on the assumption that two tetrads had germinated in close proximity, these cases were brought into harmony with the rule. In only four instances of the 81 the distribution of sexes was "abnormal" (5 females and 3 males, 1 female and 3 males, and in two cases 3 females and 1 male). The plants thus studied by Douin apparently included representatives of both *S. Michelii* and *S. texanus*. The evidence thus furnished was cited by Strasburger (1909) as proof that in *Sphæro-*



FIG. 9. A female plant of *Sphærocarpos Donnellii* from a greenhouse culture; sporophytes are present within the swollen bases of several of the involucre, as at *s, s*, $\times 7$. FIG. 10. A portion of a similar plant in vertical section showing the relation of the sporophytes (*s, s*) to the structures of the gametophyte. $\times 7$. FIG. 11. A group of four spores, resulting from the division of a single spore mother cell and remaining permanently attached. $\times 312$. Drawings by Miss Martha Engel.

carpos the sex tendencies, brought together in the union of egg and antherozoid and remaining united, though of course not finding expression, during the life of the sporophyte, are separated by the

reduction divisions which result in the formation of four spores from each spore mother cell—a notion strongly suggested by previous investigations of diœcious liverworts and mosses.

Spores being available in considerable numbers in my cultures, it seemed worth while to attempt to determine whether the conclusions thus arrived at for the European species hold likewise for *S. Donnellii*. Spores liberated by breaking the capsule wall in a drop of water were sown on soil June 16, 1916. Spores sown at this time of year have been found to be relatively slow in germination; the sporelings appeared during the following autumn and winter. On March 10, 1917, most of the plants that resulted having begun to produce involucre, those that had grown from the spores from two capsules were carefully examined, with the results shown in Table I. As a rule, plants of a group of four could be reason-

TABLE I.

SEX OF PLANTS GROWN FROM SPORES FROM TWO CAPSULES OF
Spharocarpos Donnellii (CULTURE PSC2B).

Spore tetrads sown June 16, 1916; plants* examined March 10, 1917.

2 females, 2 males	3 groups
2 females, 1 male	2 groups
1 female, 2 males	1 group
1 female, 1 male	1 group
2 females,	1 group
2 males	3 groups
1 female,	1 group
1 male	1 group
1 female, 2 males, 1 ?	3 groups
2 ?	1 group
4 ?	1 group
3 females, 1 male,	1 group
4 females, 2 males,	1 group
2 females, 3 males, 2 ?	1 group
3 females, 2 males, 3 ?	1 group
Totals: 30 females, 32 males, 14 (?).		

ably assumed, because of their contiguity, to have come from the spores of a single tetrad. Sometimes, however, the plants in a group were sufficiently separated to make their relationship, though probable, open to question. Isolated plants, or groups of two or three, evidently indicated that one or more spores had failed to

germinate, or that the sporelings growing from them had died. A question mark (?) in the table indicates that no involucre had yet appeared upon the plant in question, so that its sex could not be determined.

It is plain that all the cases shown in Table I., with the exception of the last four, harmonize with the expectation of two females and two males. The last three cases can be made to harmonize by the assumption made by Douin, namely, that two spore tetrads germinating in close proximity may give rise to a group of eight plants (or fewer), which will be expected to include four females and four males. There remains one case (of three females and one male) which does not agree with the expectation, unless it be assumed to represent the four survivors of a group of eight.

However, it appeared from this study, as well as from a more extended observation of the behavior of plants in culture, that the question of the sex potentialities borne by the spores can be finally settled only by the use of more exact methods. The sources of possible error are at least three: the impossibility of determining with certainty just which plants have come from the spores of a single tetrad; the multiplication of plants as a result of their branching and the separation of the branches as independent plants, either by accident or by the death of the older portions; and the production of new plants by regeneration. All these sources of error are likewise present in the observation of plants growing in nature, although the second is perhaps less important under these conditions because of the slower growth out of doors. It is thus quite possible that Douin's "abnormal" cases may not be real exceptions to the general rule; although, on the other hand, it must be admitted as at least conceivable that in an occasional instance an error from one of the causes mentioned might make a really aberrant case seem to agree with the rule. The possibility of a modification of the apparent sex ratio as a result of branching or of regeneration has recently been recognized by Douin (C. and R. Douin, 1917).

Attempts to develop better methods of studying this question led to the sowing of isolated tetrads on various substrata. Difficulties have been met with in securing favorable conditions for germination and in preventing contamination, and the results thus

far are small. In one culture, however, a sufficient number of germinations were obtained to make the results of some value (Table II.). In this case 24 tetrads, all from a single capsule, were sown on filter paper which was moistened with a nutrient solution made according to the formula given by É. and É. Marchal (1907),

TABLE II.

SEX OF PLANTS DERIVED FROM SPORES FROM ONE CAPSULE OF
Sphærocarpos Donnellii (CULTURE R19).

Spore tetrads sown separately on moistened filter paper March 17, 1917;
sporelings separated and removed to pots of soil May 25 to June 1, 1917.

2 females, 2 males	1 group
2 females, 1 male	2 groups
1 female, 2 males	1 group
1 female, 1 male	1 group
2 females,	1 group
1 female,	1 group
1 male	1 group
1 female, 1 male, 1 ?	1 group
1 female,	2 ?	2 groups
1 female,	1 ?	1 group
2 males, 1 ?	1 group
1 male, 1 ?	3 groups
2 ?	1 group

Totals: 15 females, 14 males, 12 (?).

placed in a petri dish, and sterilized before the spores were sown. The sporelings were removed from the filter paper while they were still very small and while their attachment to the spore wall could still be distinguished; there could thus be no question as to the origin of the plants of a group, and their youth precluded the possibility of a previous multiplication by vegetative means. The sex of the plants was determined, so far as this could be done, at the time of their removal from the filter paper, and each was transplanted to a separate pot of soil. In the cases in which the plants continued to live after transplanting, observations as to their sex were made after further development. Some of the plants died after transplanting and before producing involucre, and these are the ones whose sex is indicated as doubtful in the table.

Although more extended observations are desirable, it seems

quite clear from these results that the general conclusion arrived at by Douin and Strasburger for the species of *Sphaerocarpos* considered by them applies also to *S. Donnellii*—namely, that the differences which distinguish female from male plants result from differences in the spores that are to give rise to them, and that of the spores formed by the division of a single mother cell two bear female potentialities only and two male potentialities only. It seems probable, too, that exceptions to this rule, if any occur, are less frequent than might be thought from Douin's observations—his apparently aberrant cases probably having resulted from some of the causes of error already noted. It is not intended to be suggested, however, that positive exceptions to the rule will not be found. The possibility of such exceptions will be discussed on a later page. The occurrence or non-occurrence of an exceptional distribution of sex characteristics, and the proportion of such occurrences, if any, are evidently to be determined by the observation of thousands of plants rather than of a few score.

It will be noticed from Tables I. and II. that the number of plants certainly referable to the respective sexes is substantially equal: in the one case 30 females and 32 males, in the other 15 females and 14 males. Thus it appears that so far as power of germination is concerned there is no marked difference between female-producing and male-producing spores. This fact is striking in view of the great difference in favor of the female plants in rate of growth and in power of resistance to unfavorable conditions. Apparently this difference, which for want of a better word may be spoken of as one of "vigor," does not exist, or at least come to expression, as between the spores, but becomes apparent at some time after germination and during the development of the gametophytes.

While there seems to be no difference between the female- and male-producing spores in their capacity for germination, it remains possible that there is a difference in time or rate of germination. On this question the foregoing observations throw no light, but a suggestion is furnished by a recent set of experiments in which an attempt has been made to study this particular point. On December 27 and 28, 1918, 67 tetrads from two capsules were sown in as many pots of soil, one tetrad to a pot. Germination has been slow, and

additional sporelings are still occasionally appearing. However, the slowness of germination has given opportunity for the entrance of blue-green algæ, whose presence in quantity either checks further germination or results in the death of the young sporelings while very minute. The germinations observed in this series have resulted as follows:

In 12 pots, 1 female sporeling;

In 4 pots, 1 male sporeling;

In 1 pot, 1 of undetermined sex;

In 1 pot, 1 female followed by 1 female;

In 1 pot, 1 male followed by one male, and this by one of undetermined sex (the latter possibly, though apparently not, a regenerated shoot);

In 1 pot, 2 males appearing nearly or quite simultaneously;

In 1 pot, 1 female followed by 2 of undetermined sex.

Thus in 14 cases a female-producing spore seems to have been first to germinate; in 6 cases, a male-producing spore. While too fragmentary to furnish a basis for a conclusion, these figures suggest that there may be a difference in rate of germination in favor of the female-producing spores.

THE CHROMOSOMES OF SPHÆROCARPOS.

Sphærocarpos suggested itself as a favorable plant in which to look for a possible chromosome difference between the sexes, both because of its marked sexual dimorphism and because of the strong evidence of a relation between chromosome reduction and the separation of sex potentialities. So far as published records show, the only previous attempt to study the chromosomes of *Sphærocarpos* was by Strasburger (1909), who reports that neither in the nuclear divisions in the spore mother cell nor in the structure of the nuclei formed by these divisions did he find any evidence of the separation of structures that could be interpreted as the bases of sex differentiation.

In April, 1914, through the courtesy of Professor Mangin and of M. Capus of the Muséum d'histoire naturelle of Paris, I was enabled to locate living plants of *Sphærocarpos* (probably of *S. Michelii*) in the neighborhood of Bois-le-Roi. Some of this mate-

rial was fixed and studied in Professor Mangin's laboratory. The amount available was too small and the stage of development for the most part too advanced to furnish definite results; but it gave an opportunity for acquiring a familiarity with the plants and for testing methods of fixation. When greenhouse cultures of *S. Don-*



FIGS. 12 AND 13. Chromosome groups from female gametophytes of *Sphærocarpos Donnellii*: Fig. 12, from a basal cell of an archegone; Fig. 13, from the basal part of a young archegonial involucre; *x*, the large chromosome. FIGS. 14 AND 15. Chromosome groups from male gametophytes of *S. Donnellii*: Fig. 14, from the wall of a young antherid; Fig. 15, from a projecting superficial cell of the apical meristem, possibly an antheridial initial; *y*, the small chromosome. $\times 3800$.

nellii were later obtained in vigorous condition, the cytological study was renewed. Some of the results of this study have already been briefly reported (Allen, 1917*b*).

Figs. 12 and 13 show typical chromosome groups from cells of the female gametophyte; Figs. 14 and 15, corresponding groups from cells of the male gametophyte. In each case, as the figures show, eight chromosomes are present. In the female, one of the eight chromosomes (*x*, Figs. 12, 13) is much longer and thicker than any of the others. The other seven differ in length among themselves. It is probable that all the individual chromosomes of different cells can be identified by their length; but since in sections the chromosomes lie at various angles and thus some of them are always foreshortened in the camera lucida drawings, it will require the assembling and study of a considerable number of figures showing

the chromosomes in favorable positions to make such identification reasonably certain. However, in all of the numerous division figures that have been seen in various parts of female plants the single large chromosome is present and conspicuous.

The chromosome groups of the male contain no element at all like the large chromosome of the female. Seven of the chromosomes of the male, varying in length among themselves, seem to correspond to the seven smaller ones of the female. The eighth chro-

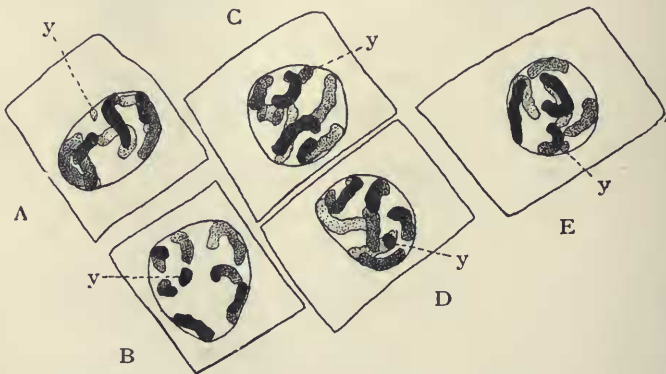
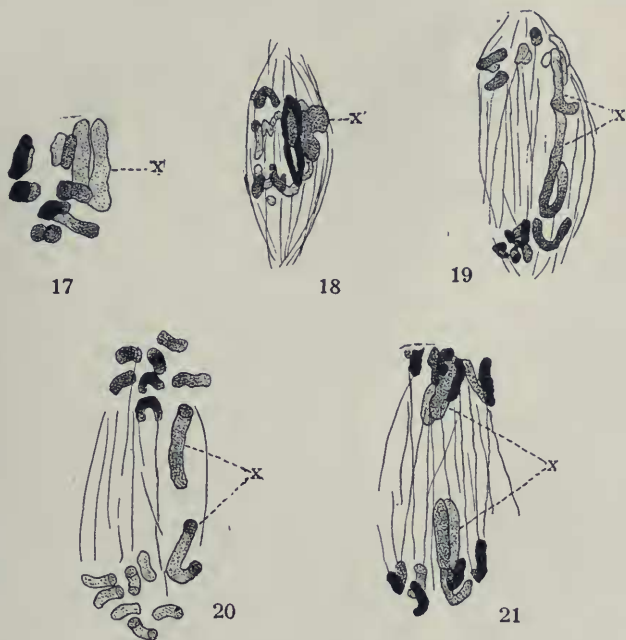


FIG. 16. A group of cells from a developing antheridium; nuclei in late prophase; *y, y*, the small chromosome. $\times 3800$.

mosome (*y*, Figs. 14, 15, 16) is very small. This small chromosome is not always easily distinguishable. In the group shown in Fig. 14, for instance, the body *y* is very lightly stained; the same is true of the body similarly identified in cell *A*, Fig. 16. However, in the majority of cases, as in cells *B*, *C*, *D*, and *E*, Fig. 16, and in the cell shown in Fig. 15, in which the chromosomes are unusually widely scattered, this small element is stained like the other chromosomes and is plainly one of them. It appears certain, therefore, that a very small chromosome in the male in some way corresponds to, or replaces, the very large one of the female.

Figs. 17 to 21 show stages in the division and separation of the chromosomes in dividing cells of the female. In the cell represented in Fig. 17 the chromosomes or most of them, including the large one, are longitudinally split. Figs. 18 to 20 illustrate a peculiarity in the behavior of the large chromosome—namely, that its daughter

halves are the last to separate in the metaphases (Fig. 18), and that they lag behind the other daughter chromosomes in their passage to the poles of the spindle (Figs. 19, 20). Even at the late stage shown in Fig. 21, the large daughter chromosome moving toward the lower pole of the figure is still a little in the rear of its fellows, which have reached the spindle pole. In this respect the behavior of the large chromosome recalls that of the X-chromosome in the heterotypic divisions of certain insects, and in some cases at least



FIGS. 17-21. Stages in nuclear division in the female gametophyte: Fig. 17, a late prophase, the chromosomes showing longitudinal splitting, in a cell of the calyptra; Fig. 18, a metaphase in an involuclral cell; Fig. 19, an anaphase in an involuclral cell; Fig. 20, a later anaphase in a young thallus lobe; Fig. 21, a diaster in a cell at the base of a young lobe; *x*, the large chromosome. $\times 3800$.

in the homœotypic divisions of these animals as well. It is quite possible, to be sure, that this lagging of the large chromosome of *Sphaerocarpos* is merely a result of its proportionately great size.

Relatively few division figures have been observed as yet in the

developing sporophyte. Those which have been seen show that about sixteen chromosomes are present and that one and only one of the group is much larger than the others, being, therefore, obviously derived from the female parent. Fig. 22 represents the two anaphase groups in a dividing cell of a young sporophyte; each group shows fifteen chromosomes, one of which is the large one. The sixteenth chromosome which would be expected to appear in



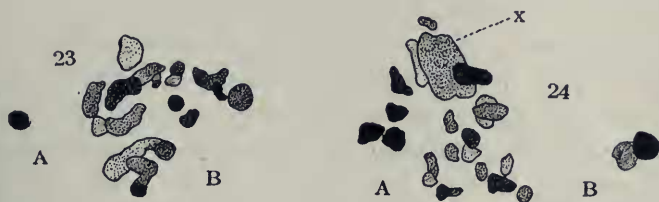
FIG. 22. Two anaphase chromosome groups in a dividing cell of a young sporophyte; *A*, the group seen at an upper, *B*, that seen at a lower, focus; *x*, the large chromosome. $\times 3800$.

each of these groups is, it seems likely, the small element derived from the male parent, which here is not seen either because of its too light stain or because it is hidden by one of the others.

The first division of the spore mother cell nucleus (the heterotypic division) has not yet been seen; but a considerable number of spore mother cells have been found in which the second (homœotypic) division was in progress. In every one of these latter cases in which the complete series of sections of a spore mother cell was present, one of the two spindles in the cell bore a large chromosome, and the other did not. Figs. 23 and 24 show polar views of the two homœotypic metaphase groups in a single spore mother cell; in the group of Fig. 24 a large chromosome (apparently longitudinally split) is present; the group of Fig. 23 includes no large chromosome. An exact count of the smaller chromosomes has not been found possible at this stage because of the irregularities in their shape and distribution. The fact that a single group is commonly represented in several sections (those chosen for Figs. 23 and 24 are unusually favorable in this respect) involves additional difficulties.

The conclusion seems warranted, from the presence of a large

chromosome on only one of the two homœotypic spindles in a spore mother cell, that in the heterotypic division the large and small chromosomes pass respectively to different daughter nuclei. This means that the separation of these two very different bodies is effected in the same division as that which, on the basis of the best cytological evidence, seems to result in the qualitative separation of other ("ordinary") chromosomes. It remains to determine from a study of the heterotypic division just how this separation is brought about.



FIGS. 23 AND 24. The two chromosome groups, seen in polar view, in a metaphase stage of the homœotypic division in a spore mother cell. The great majority of the chromosomes on the two spindles (shown in Figs. 23 *B* and 24 *A*) were in the same section; Fig. 23 *A* shows a single chromosome belonging to the group of Fig. 23 *B*, appearing in the previous section; and Fig. 24 *B*, two chromosomes (or a dividing chromosome) belonging to the group of Fig. 24 *A*, and appearing in the following section; *x*, the large chromosome. $\times 3800$.

Of the four nuclei formed in the spore mother cell, two sister nuclei, and therefore the two spores into which these nuclei pass, receive a large chromosome each; the other two nuclei (and spores) receive a small chromosome each. Since the large chromosome is always present in the cells of the female and never in those of the male, it follows that a spore receiving a large chromosome must develop, if it develops at all, into a female gametophyte, and that a spore receiving a small chromosome must develop, if it develops at all, into a male gametophyte.

THE CHROMOSOMES AND SEX CHARACTERS.

What I have hitherto referred to respectively as the "large" and the "small" chromosome of *Sphærocarpos* are so closely similar in appearance and behavior to the sex chromosomes of various

animals—especially of such insects as *Lygæus* and *Euschistus* (Wilson, 1906, 1912; Montgomery, 1911)—that in the remainder of the present discussion they will be designated, as commonly are the apparently corresponding bodies in animals, the “X-” (large) and “Y-” (small) chromosomes. This terminology is borrowed for the sake of simplicity, in spite of the fact that it may require revision in the light of future investigations.

Whatever may later appear in this respect, at present the problem of the relation of the X- and Y-chromosomes to the complexes of characters that distinguish male from female seems to be similar in its broadest terms in *Sphærocarpos* and in those animals whose possession of sex chromosomes has been demonstrated. The suggestion of an underlying similarity, however, does not involve maintaining that the precise relation of these particular chromosomes to certain hypothetical sex-determining factors is identical in all organisms in which X- and Y-chromosomes are found. Indeed, there appear at present to be, as regards the relation between special chromosomes and characters distinctive of sex, three quite different types of cases:

(a) Those in which, in terms of the chromosomes, the female is homozygous because it possesses what Wilson (1909) has called two “X-elements”—the X-element in different instances being a chromosome or a group of chromosomes; and the male heterozygous because it possesses either one X-element only, or one X- and one Y-element; the Y-element, if present, sometimes consisting of one body, sometimes of a group. To this class are now referable a large number of animals representing several phyla.

(b) Those in which the male is homozygous, possessing two X-elements, and the female heterozygous, possessing one X-element only, or one X- and one Y-element. This class, apparently smaller than the first, is established upon the basis of suggestive cytological observations which in no case as yet cover the whole life history, and of a larger amount of evidence derived from experimental breeding.

(c) Those in which the female possesses one X-element (or chromosome), the male one Y-element, and in which the diploid generation (the sporophyte) is heterozygous in terms of the chro-

mosomes although phenotypically asexual. To this class can be assigned at present with certainty only two species of *Sphærocarpos*: *S. Donnellii*, discussed in the present paper, and *S. texanus*, in which Miss Schacke (1919) has recently found a condition as to chromosomes similar to that in *S. Donnellii*. It may be hazarded, however, that here belong also the dioecious mosses upon which the epoch-making studies of the Marchals (1907, 1909, 1911) were made; the heterozygous sporophytes of these mosses were induced to give rise by regeneration to diploid and hermaphroditic gametophytes.

The differences in the apparent relations of the chromosomes in question in the organisms representing these three classes are brought out strikingly by the facts that in class *a* two X-elements are necessary to the appearance of femaleness, but only one X-element (with or without a Y-element) to that of maleness; that in class *b*, two X-elements are requisite to the appearance of maleness, and only one X-element (with or without a Y-element) to that of femaleness; whereas in class *c* the presence of one X-element means femaleness, that of one Y-element maleness, and the presence of both, under ordinary conditions, is correlated with non-sexuality.

Another difference, at least as between the plants of class *c* on the one hand and the animals of class *a* on the other, lies in the apparent lack of function in heredity of the Y-chromosome or -element in animals, as shown by its utter absence in many forms and by the genetic evidence of its failure when present to influence the transmission of sex-linked characters; although Bridges' (1916) evidence in this connection should be cited, namely, that the absence of the Y-chromosome in *Drosophila*, while not affecting the apparently normal development of the male animal, does result in sterility. In *Sphærocarpos*, however, there is no reason at present for considering the Y-chromosome to be in any sense a functionless body. Its presence seems, as will be indicated more fully in a later paragraph, to be related to the appearance of definite characters in just as positive a way as is the presence of the X-chromosome. And in the normally dioecious mosses studied by the Marchals, heterozygosis in the diploid gametophyte manifested itself by the appearance of both male and female characters.

The visible differences between male and female plants of *Sphærocarpos* fall naturally into two categories:

(a) Differences in rate of growth and in the size at maturity of homologous parts (compare, for example, Figs. 1 and 2, or 3 and 4), and, probably intimately connected with these, a difference in power of resistance to unfavorable conditions; in each of these respects the advantage is with the female.

(b) Differences in form and structure of the sex organs (antheridia and archegonia), and of course of the gametes themselves, as well as in the form and structure of the involucre surrounding the sex organs. These differences in structure are associated with size differences—for example, the archegonial involucre is much larger than the antheridial involucre, as well as different in form—but it does not follow that characters of size and those of form, though constantly associated and even causally related, have been brought to expression by the same chain of causative factors.

Of none of these distinctive characters, it may be noted, is there any apparent reason for suspecting that it has anything in common with the “secondary sexual characters” of the higher animals.

That the possession by each sex of its own complex of distinctive characters—hereinafter referred to for the sake of brevity as “sex characters”—constituting a constant phenotypic difference between the sexes, is the expression of a genotypic difference, can hardly, I think, be doubted; for all the available evidence indicates that *Sphærocarpos* is strictly and under all circumstances dioecious, and that an individual gametophyte possesses one and only one set of potentialities so far as sex characters are concerned. This being the assumption upon which discussion must for the present rest, it follows that the respective groups of sex characters (or their physical bases) are separately inherited by the sexual from the asexual generation through the spores, which latter are the only possible vehicle of transmission from sporophyte to gametophyte. Since two spores of each tetrad develop into plants of either sex, the conclusion already drawn by previous writers seems inevitable, namely, that the physical bases for the sex potentialities were united in the sporophyte down to the time of the formation of the spore mother cells, and that the separation of these physical bases occurred in the

course of the divisions which formed the spores. The invariable connection of the X-chromosome with femaleness and of the Y-chromosome with maleness seems to prove a causal relation of some sort between the presence of either chromosome and the appearance of the characters of the corresponding sex. The further logical step seems likewise inevitable, although admittedly it raises questions of considerable difficulty—the conclusion, namely, that it is the presence of an X- or of a Y-chromosome in the cells of a particular plant which determines the appearance in that plant of the characters of the corresponding sex. This essentially corresponds with the hypothesis which the students of the sex chromosomes of animals seem in general to have adopted—although naturally the form of the statement, as of the precise fact, is different for an organism whose sexual generation is diploid rather than haploid.

The facts in the life of *Sphaerocarpos* that seem to force this conclusion upon us are, first, that, as already noted, the physical bases for the sex characters must have been separated in the course of the divisions in the spore mother cell and distributed among the four resultant spores; and second, that the only structures that are shown to have been so separated and distributed are the X- and Y-chromosomes—the four spores being to all appearances alike in all other points of structure, form, and size. The difficulty of imagining how the sex chromosomes can function in impressing sex characters upon the plant—a difficulty which, however, is but of the same order as that which confronts the very widely accepted theory of the function of the chromosomes in inheritance in general—will continue to breed a healthy skepticism and will stimulate the attempt to find another basis for sex inheritance; but at present this hypothesis of the significance of the sex chromosomes seems the only intelligible one and the one therefore upon which further investigations must rest.

The question then arises as to how the sex chromosomes of *Sphaerocarpos* can be conceived as exercising their controlling influence. Apparently this question may be answered in different ways as regards the two categories of sex characters already mentioned.

The notion suggests itself at once that the differences in size and

in rate of growth of the respective gametophytes may well result from the difference in bulk of chromosome substance present in the cells of female and male respectively. The size of the X-chromosome is such that the chromosome group of the female exceeds in bulk that of the male, at a rough estimate, by perhaps fifty per cent. In view of this difference, and of the differences in cell size which in many cases have been shown to result from differences in chromosome number—as, for example, between haploid and diploid moss gametophytes (É. and É. Marchal, 1909), between haploid

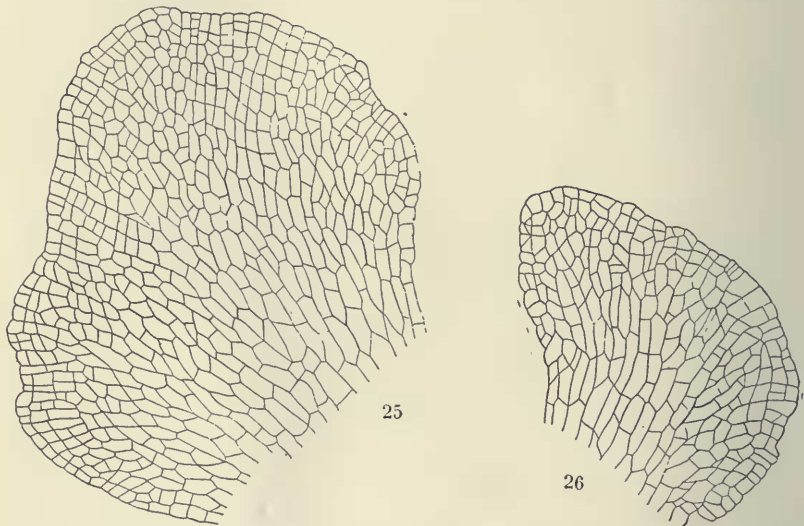


FIG. 25. A typical fully developed lateral lobe of a female gametophyte. FIG. 26. A similar lobe of a male gametophyte, drawn to the same scale. Both from greenhouse cultures of *Sphærocarpos Donnellii*. $\times 36$.

and diploid *Spirogyra* cells (Gerassimow, 1901), and between the ordinary and *gigas* forms of tomato and nightshade (Winkler, 1916), it would not have been surprising to find a marked difference in cell size between homologous members of the male and female gametophytes of *Sphærocarpos*. However, some measurements made to test this possibility showed that in corresponding parts, such as the lateral lobes, the range of cell sizes is substantially the same in the two sexes. This fact appears clearly in the camera lucida drawings of typical mature lobes from a female and a male

plant respectively (Figs. 25, 26), and of mature archegonial and antheridial involucre (Figs. 27, 28). These figures, while not negating the possibility of a small difference between the sexes in average cell size—a possibility to be tested only by a great number of measurements—demonstrate that such differences, if they exist, are negligible as compared with the difference either in total chromosome volume or in the total surface area of the chromosomes; and, what is more significant from the present point of view, that

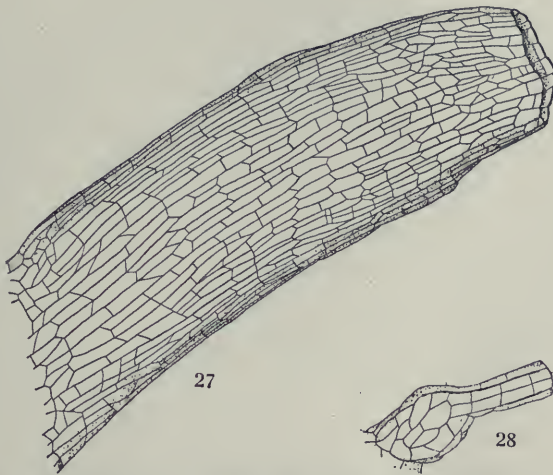


FIG. 27. A typical fully developed archegonial involucre. FIG. 28. A typical fully developed antheridial involucre, drawn to the same scale. Both from greenhouse cultures of *Sphærocarpos Donnellii*. $\times 36$.

such possible differences in cell size play no important part in bringing about the marked differences in size of homologous organs. On the other hand, as the figures referred to demonstrate, the characteristic differences in size between male and female plants result from the presence of a much greater number of cells in corresponding parts of the female. Since these size differences appear between plants of the same age, it follows that cell growth and division go on more rapidly in the female than in the male. If, therefore, the size and related differences between plants of opposite sex are determined by the difference in chromosome bulk, this quantitative difference produces its ultimate visible effects by means of its

influence on the rate of cell growth and of cell division. That this is the case seems conceivable and indeed probable, because the overwhelming evidence of an influence of the chromosomes on inheritance points to the exertion of this influence largely, at least, through a determination of the rate and nature of constructive metabolism; and one effect of a modification of the rate of metabolism would of course be an increase or decrease in the rate of cell growth and thus also in the rate of division.

But if such a simple quantitative explanation can be adduced to account for the influence of the sex chromosomes on the one class of sex characters, it cannot with equal ease be made to account for the characters of the second category—those which concern the form and structure of gametes, of sex organs, and of involucre.

It is true that, in connection with the observed facts in animals, several writers have suggested the possibility of a "quantitative theory" of sex determination, which would make all primary sex characters the expression of the amount or degree of activity of the chromosome material present. Without entering into the discussion of the validity of such a theory in the case of the metazoa, it seems quite impossible of application to the class of characters under consideration in *Sphærocarpos*. For one thing, it is to be remembered that in this plant, differently from the condition so common in the higher animals as well as in the dioecious seed plants, there are not two sets of reproductive structures, one functional, the other rudimentary but conceivably capable (certainly capable in many seed plants) of a normal development under particular conditions. In such a case the stimulus leading to the functional development of one set of structures or the other might conceivably result from the presence of a greater or less quantity of particular nuclear substances. On the contrary, the male plant of *Sphærocarpos* shows no trace of archegonia or of archegonial involucre; the female plant shows no trace of antheridia or of antheridial involucre. Nor can the differences between male and female structures be explained by modifications of cell size operating differently in different cell axes. Factors of some sort must be supposed to be at work which determine, very differently in the two sexes, the planes of successive cell divisions; which modify, also in very different ways, the nucleo-

cytoplasmic ratio; and which determine in the one case the considerable but relatively slightly specialized development of the egg, and in the other the remarkable metamorphosis of the androcyte into the antherozoid. This is but a partial analysis of the nature of the processes whose causes are to be explained, but it is sufficient to illustrate the nature and complexity of the problem.

Perhaps it is not safe to say more at present than that the appearance of the sex characters falling within the form-structure category are to be ascribed to factors which in some way are dependent upon, carried by, or inherent in, the X- and Y-chromosomes—the word “factor” here being used in its ordinary, not in a technical Mendelian sense. Nothing in our present knowledge of the mechanism of inheritance in *Sphærocarpos* would justify us in holding that the factors here in question are of the nature of those which are postulated by any particular theory of heredity. The conclusion, if it can be so called, to which we are for the present led, is therefore that one category of sex characters is reasonably explainable by the difference in mass between the sex chromosomes; and that those of a second category seem to result from some other, but unknown, specific peculiarities of the same chromosomes.

On the analogy of the irregularities which, it seems well established, occur, though rarely, in the distribution of the sex chromosomes during the reduction divisions in certain animals, it is perhaps to be expected that irregularities more or less like these will be found in *Sphærocarpos*. The occurrence of “non-disjunction,” for example, might lead to the formation of a tetrad two of whose spores have both an X- and a Y-chromosome each, the other two spores possessing neither. It would be idle to speculate as to the effect of such a distribution upon the viability of the spores or upon the sexuality or sterility of the resulting plants; but it is plain that the result might well be a disturbance of the normal 2:2 sex ratio.

Other irregularities than non-disjunction might conceivably likewise lead to modifications of this ratio; and so it will not be surprising should a study of the results of the germination of large numbers of spores bring to light occasional exceptions to the general rule of the distribution of sex characters. It is possible that some of the

apparent exceptions found by Douin were the result of an aberrant behavior of the sex chromosomes; but reasons have already been given for doubting whether these exceptions were other than apparent. In the examination of spore tetrads I have come across a few instances of spores joined in twos rather than in fours; and other instances in which a group consisted of two spores of normal size and one or two very small. Such conditions may result from an irregular distribution of the chromosomes; but they may equally well or perhaps better be explainable by the death or accidental injury or destruction of one or two spores after the completion of the division of the mother cell.

The existence of definite sex chromosomes having been established for two species of *Sphærocarpos*, it is to be expected that similar bodies will be found in other plants. The most promising organisms for such investigations are probably the other dioecious bryophytes, especially those with a marked sexual dimorphism. Next would come perhaps some of the dioecious algæ. That previous searches for sex chromosomes in plants have been fruitless has been largely because they dealt chiefly with dioecious seed plants. Enough of these have now been examined to make it quite plain that no visible chromosome difference is to be expected as between the staminate and the pistillate individuals of any species. This negative result is quite in harmony with what we now know regarding *Sphærocarpos*, because the distinction between staminate and pistillate sporophytes is of a quite different sort from that between male and female gametophytes; and if anywhere in the seed plants a chromosome difference is to be looked for exactly comparable with that described in the present paper, it must be between the micro- and the macrogametophytes. Evidence as to the possible existence of such a difference is still meager; but such evidence as does exist is, it must be admitted, of a negative character.

In a previous paper (Allen, 1917a) I have referred to Hirasé's (1898) description of a cytological difference between the two antherozoids produced by the same microgametophyte of Ginkgo, which might be imagined to be the basis of the dioecism of the sporophytes of this species. This difference consists in the presence of a

conspicuous cytoplasmic body in one antherozoid and its absence in the other. The difference is brought about, not during chromosome reduction, but in the final division in the microgametophyte, and has no apparent parallel, so far as I know, in the cytology of any other diœcious seed plant. However, little or nothing is known regarding the corresponding division in other diœcious plants.

Correns' (1907) well-known experimental studies of sex inheritance in *Bryonia* seemed to show the existence in *B. dioica* of male gametes of two kinds with reference to sex potentialities. Correns, as well as most of those who have attempted an explanation of his results, has assumed that this involves the existence of two kinds of pollen grains, so strong has seemed the probability that the segregation of any particular hereditary factors is brought about by the reduction divisions. It is, however, quite possible to assume that, so far as sex potentialities are concerned, the pollen grains of *Bryonia* are alike, and that the segregation of sex potentialities occurs in the division of the generative cell within the pollen grain or pollen tube. This suggestion, though based only upon the obscure phenomenon recorded for Ginkgo, is perhaps worth testing, in view of the failure of all attempts to find other visible bases for the separate inheritance of staminate and pistillate characters.

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